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Captan-Treated Seed Corn in Alcohol Production

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Utilization of surplus captan-treated corn has long been of concern to seed producers. Results of this study show that it can be used effectively as a substrate for ethanol fermentation. When captan-treated seed corn and untreated corn were used in 10-L fermentations with recycled distillers' solubles, higher ($P < 0.10$) alcohol production resulted from captan-treated corn (91.8%) than from untreated corn (84.0%). Chicks fed diets containing 10% and 20% distillers' dried grains (DDG) from the treated corn fermentations had higher gain:feed ratios at day 7 than did chicks fed diets containing DDG from control fermentations. No significant differences in any performance measurement occurred at day 14, but chicks fed DDG from the treated corn fermentations had the highest rates of gain and gain:feed ratios. Practical use of this procedure will probably require approval by appropriate regulatory agencies.

The controversy over the method for disposal of surplus captan-treated seed corn has been ongoing for a number of years. Captan [*N*-[(trichloromethyl)thio]tetrahydrophthalimide] is used on all seed corn produced (45.5 g of captan/45.5 kg of seed corn) and acts to protect the seed from fungal infestation. An estimated 1.97 billion lb of seed is treated with captan every year, with a carry-over of unused seed corn (55–110 million lb) resulting. Captan is believed to have toxic properties; this potential toxicity, when incorporated directly into animal diets, has been studied (Ackerson and Mussehl, 1955; Dowe et al., 1957; Theuninck et al., 1981) with varying results. Evidence of harmful effects on humans has also been inconsistent (Environmental Protection Agency, 1980). Until recently, the approved method of disposal was by burial. However, the Environmental Protection Agency has changed the method of disposal of the treated corn; it now allows feeding to cattle or hogs if the captan level is below 100 ppm after either roasting or washing (*Fed. Regist.*, 1981). Washing the seed corn with water degrades captan to sulfur, chloride, and 4-cyclohexene-1,2-dicarboximide (Wolfe et al., 1976). If a strong base is used as a wash, the primary breakdown product is also 4-cyclohexene-1,2-dicarboximide (Coats and Dahm, 1980).

One use for surplus captan-treated corn could be as a fermentation substrate for alcohol production, with recovery of the solid portion (distillers' dried grain, DDG) as an animal feed. To reduce the cost of concentrating solids after a fermentation, the spirits industry recycles part of the distillers' solubles (up to 20%) back into the makeup water of the next fermentation. Recycling also seems to accelerate yeast growth and fermentation. Ex-

tensive recycling has not won widespread approval, but studies (Ronkainen et al., 1978; Wall et al., 1983) have shown alcohol yields did not diminish even when 70% or more of the distillers' solubles were recycled. Also, researchers have used ethyl alcohol as a wash to remove the captan, fermented the washed corn to produce alcohol, and then used this alcohol for the next wash (Steinberg et al., 1982).

This report describes a study of (1) the fermentability of captan-treated seed corn while recycling the distillers' solubles in increasing amounts (0, 25, 50, 75, and 100%) and (2) the performance of chicks fed a diet containing the spent grains from the fermentations.

MATERIALS AND METHODS

Captan-treated seed corn (obtained commercially) and yellow-dent field corn were ground through a 0.063-in. screen with a Fitzpatrick Homoloid Model J. T. mill. Each type was mixed thoroughly before use.

Fermentations of both captan-treated seed corn and untreated field corn were run in tandem using 20-L stainless steel fermentors equipped with stirrers and temperature control jackets. Ground corn (2945 g of captan-treated or field corn) was added to 6.25 L of distilled H₂O (some or all of this water would be substituted with distillers' solubles when recycling). The pH was adjusted to 6.2, and 7.5 mL of a bacterial α -amylase (Taka-therm, Miles Laboratories) was added for liquefaction. The fermentors were then heated to 90 °C with stirring and held for 1 h. At the end of this period, 1950 mL of distilled H₂O (again, some or all might be recycled distillers' solubles) was added and the temperature of the fermentors was dropped to 60 °C. The pH was adjusted to 4.0 and 22.5 mL of a fungal glucoamylase (Diazyme L-100, Miles Laboratories) was added for conversion of dextrins to glucose. The fermentors were held at 60 °C for 2 h, after which they were cooled to 32 °C and the pH was adjusted to 5.0. The pH adjustments were achieved with either dilute NaOH or dilute HCl. A yeast inoculum was added (5% v/v) and

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Table I. Diets Used in Chick Bioassay Incorporating Distillers' Dried Grains from Captan-Treated Seed Corn and Field Corn Fermentations

ingredients	% for diet no.				
	1	2	3	4	5
ground corn	56.51	48.98	48.98	41.45	41.45
corn oil	5.23	6.07	6.07	6.90	6.90
soybean meal	23.94	20.73	20.73	17.52	17.52
fish meal	10.00	10.00	10.00	10.00	10.00
corn gluten meal	2.00	2.00	2.00	2.00	2.00
alfalfa meal	1.00	1.00	1.00	1.00	1.00
limestone	0.62	0.63	0.63	0.65	0.65
dicalcium phosphate	0.59	0.49	0.49	0.38	0.38
vitamin premix ^a	0.10	0.10	0.10	0.10	0.10
DL-methionine	0.01				
DDG		10.00		20.00	
DDG, captan treated			10.00		20.00

^a Vitamin premix provided per kg of diet: vitamin A, 4400 IU; vitamin D₃, 1000 ICU; vitamin E, 11 IU; vitamin B₁₂, 0.01 mg; riboflavin, 441 mg; d-pantothenic acid, 10.0 mg; niacin, 22.0 mg; menadione sodium bisulfite, 2.33 mg.

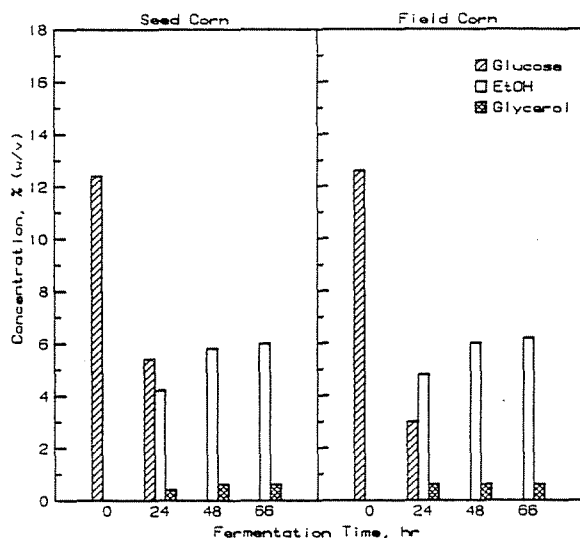


Figure 1. Glucose utilization and EtOH and glycerol production from standard fermentations of captan-treated seed corn and field corn; no recycle of distillers' solubles.

allowed to ferment for 66 h at 32 °C with stirring.

The inoculum consisted of 9 g of Fermivin dry yeast (G. B. Industries, *Saccharomyces cerevisiae*) added to 500 mL of a yeast-malt medium (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1.0% dextrose) and incubated for 24 h at 32 °C and 150 rpm. The fermentation was allowed to continue for 66 h, at which time the alcohol was distilled off with steam and the distillers' grains and distillers' solubles were separated by filtration through cheesecloth and centrifugation.

Samples were removed from the fermentors at 0, 24, 48, and 66 h. After being filtered, samples were analyzed for ethanol by using a Varian 3700 gas chromatograph equipped with a 6-ft Porapak Q column operated at 190 °C. Glucose and glycerol analyses were done by using a Waters high-performance liquid chromatography system and a Bio-Rad HPX87H column (300 × 7.8 mm) with 0.01 N H₂SO₄ solvent at 45 °C and a Waters R401 differential refractometer detector.

The distillers' grains were dried and used for chick-feeding studies, which were conducted for 14 days with 8-day-old New Hampshire × Columbian crossbred male chicks (average initial weight, 83 g). Chicks were housed in a stainless steel Petersime brooder maintained at 33 °C.

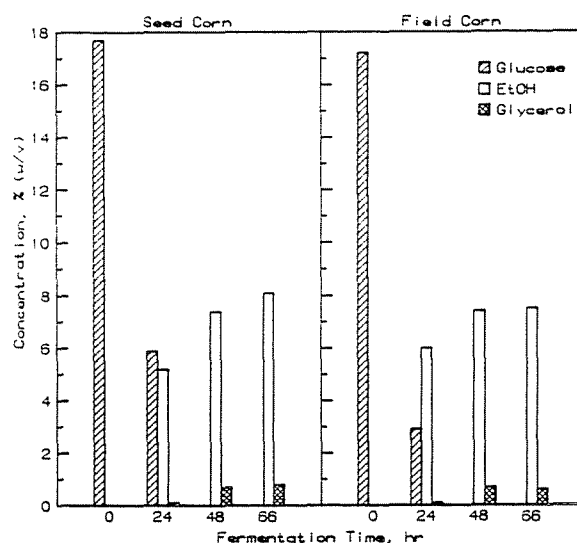


Figure 2. Glucose utilization and EtOH and glycerol production from standard fermentations of captan-treated seed corn and field corn; 25% recycle of distillers' solubles.

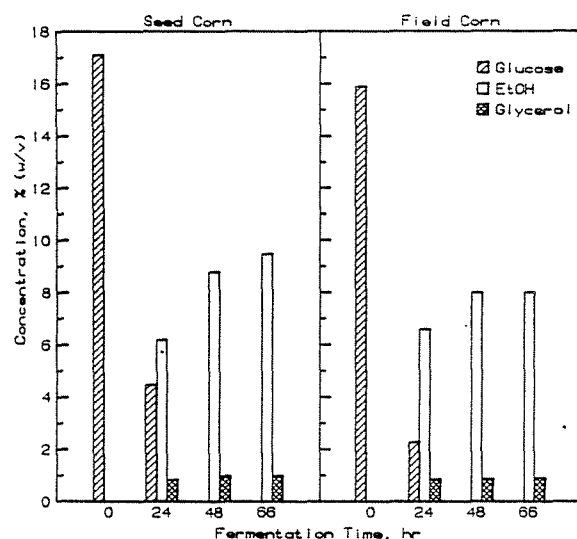


Figure 3. Glucose utilization and EtOH and glycerol production from standard fermentations of captan-treated seed corn and field corn; 50% recycle of distillers' solubles.

Chicks were allotted in a completely randomized design with three pens of five chicks per pen. Diets (Table I) were formulated to meet minimum amino acid requirements. Treatments consisted of distillers' dried grains (DDG) incorporated at 10% or 20% of the diet, both with or without captan treatment. The chicks were weighed weekly and the amount of feed remaining was recorded and disposed of. Water was supplied ad libitum. Criteria measured included growth rate and feed consumption, allowing gain:feed ratios to be calculated.

RESULTS AND DISCUSSION

Throughout this discussion, when we refer to seed corn or field corn we are referring to the fermentation of each. Also, when we refer to a percent recycle, we are referring to that percent of the makeup water of the fermentation that is composed of recycled distillers' solubles.

Glucose utilization and alcohol and glycerol production data are shown in Figures 1–5. Except for seed corn with 100% recycle (which had 0.1% glucose remaining after 48 h), glucose concentrations were completely expended

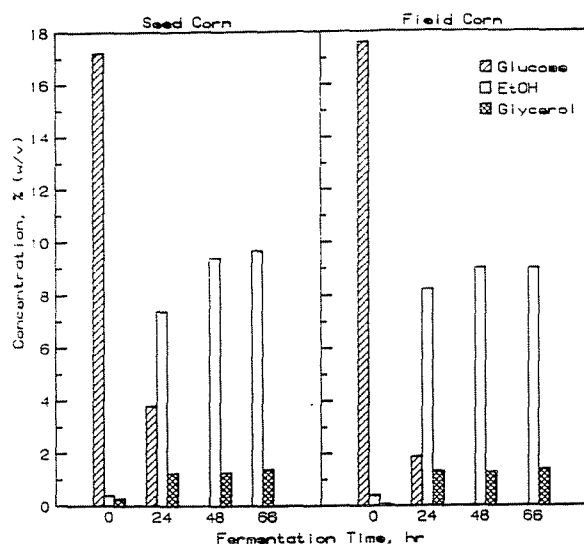


Figure 4. Glucose utilization and EtOH and glycerol production from standard fermentations of captan-treated seed corn and field corn; 75% recycle of distillers' solubles.

Table II. Alcohol Conversion Efficiency (Percent)^a of Seed and Field Corn Fermentations Using Recycled Makeup Water

recycle	field corn	seed corn
none	63.9	59.9
25%	77.3	80.2
50%	82.5	94.1
75%	92.8	96.9
100%	83.5	95.9
recycle treatment means ^b	84.0	91.8

^a Alcohol conversion efficiency is the assayed value of ethanol produced as a percentage of the theoretical ethanol yield possible, which included standardization for a difference in starch and moisture content of the corn. ^b A difference of 7.8 between recycle treatment means approaches significance at the 0.05 level ($P < 0.10$). Individual conversion efficiency values are not significantly different.

within 48 h for all of the fermentations. Alcohol conversion efficiency (Table II) was higher for seed corn than for field corn when recycling was used. Alcohol production from field corn increased from 64.7% with no recycle to 93.7% with 75% recycle. This level declined to ~84% with 100% recycle. Alcohol production from seed corn increased more rapidly than it did from field corn (~94% compared to 83% using 50% recycle) and remained at ~96% with both 75% and 100% recycle.

The performance data for chicks are summarized in Table III. In all cases, chicks fed the control diet gained more slowly than chicks fed distillers' dried grains (DDG), with or without captan. Overall, gains were highest during the second week of the experiment, with chicks fed 20%

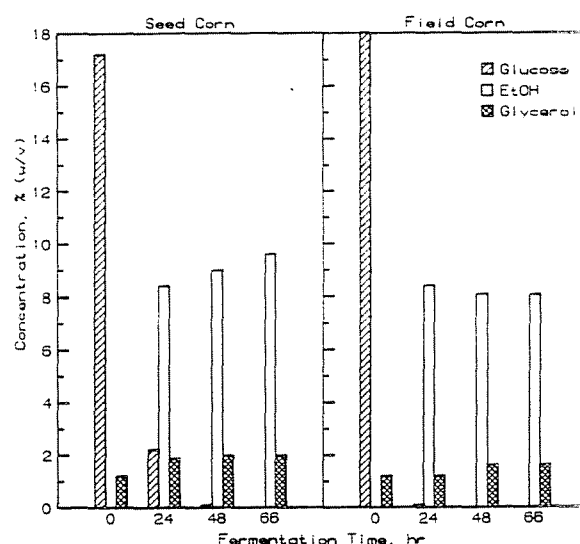


Figure 5. Glucose utilization and EtOH and glycerol production from standard fermentations of captan-treated seed corn and field corn; 100% recycle of distillers' solubles.

captan-treated DDG gaining at the fastest rate (21.1 g/day). Although values were not significantly different, chicks fed captan-containing DDG tended to have faster rates of gain than those fed DDG from the control. Feed intake did not differ among treatments. Only during the first week of the experiment were gain:feed ratios of chicks fed DDG significantly ($P < 0.001$) improved over those of control-fed chicks, with those fed 20% DDG having higher gain:feed ratios than those fed the 10% level. Captan appeared to have no effect during week 1, but values for week 2 and the combined 14-day data indicated increased gain:feed ratios with increasing levels of captan. Responses from feeding untreated DDG were similar to those from controls.

These results complement those of Theuninck et al. (1981), who showed that steers fed captan-treated grain gained faster ($P < 0.05$) and had higher ($P < 0.05$) weight gains per unit of feed. However, Ackerson and Mussehl (1955) showed that Orthocide (the active ingredient of which is captan) fed to chicks depressed gains initially compared to controls. In this latter study, captan was added directly to the diet in known quantities. In the present study, seed corn was subjected to a distillation process that might have altered the captan. Such altered captan could retain fungistatic properties and modify gut microflora to potentiate a growth response.

In conclusion, we have shown that captan-treated seed corn is an effective substrate for alcohol production and that recycling distillers' solubles from these fermentations can result in improved alcohol production. In short-term feeding trials the DDG from these fermentations can be used in chick diets without harm to the animal.

Table III. Performance of Chicks^a Fed Diets Containing Distillers' Dried Grains from Captan-Treated Seed Corn and Field Corn Fermentations

diet	weight gain, g/day			feed intake, g/day			gain:feed		
	days 1-7	days 7-14	days 1-14	days 1-7	days 7-14	days 1-14	days 1-7	days 7-14	days 1-14
1, control	15.4	19.3	17.3	21.7	32.8	27.3	0.709	0.589	0.636
2, 10% DDG	16.0	20.1	18.0	21.7	34.2	27.9	0.737	0.587	0.645
3, 10% DDG, captan	16.2	20.8	18.5	21.7	32.9	27.3	0.746	0.632	0.677
4, 20% DDG	15.9	19.3	17.6	21.0	33.1	27.1	0.754	0.584	0.650
5, 20% DDG, captan	16.3	21.1	18.8	21.7	33.4	27.5	0.754	0.635	0.682
SEM ^b	0.28	0.73	0.43	0.30	1.11	0.65	0.005	0.20	0.01

^a Means of triplicate groups of five chicks. ^b Standard error of means.

Registry No. Captan, 133-06-2; ethanol, 64-17-5.

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